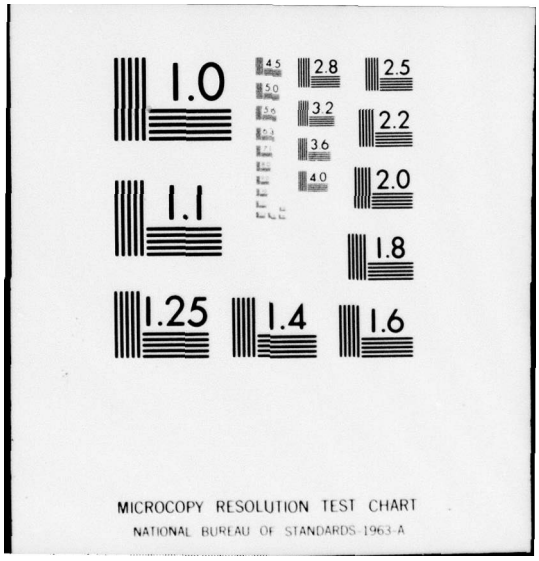
AD-A036 986 ARMY MOBILITY EQUIPMENT RESEARCH AND DEVELOPMENT COMM--ETC F/G 11/5
CHEMICAL ANALYSIS FOR CHITIN AS A MEASURE OF FUNGAL INFILTRATIO--ETC(U)
DEC 76 G ERNST, D A EMERIC, S LEVINE

UNCLASSIFIED MERADCOM-2200 NL

1 OF 1
ADA036986

END

DATE
FILMED
4-77


1.0
1.1
1.25
1.4
1.6
1.8
2.0
2.2
2.5
2.8
3.2
3.6
4.0
4.5
5.0
5.6
6.3
7.1
8.0
9.0
10.0
MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A

AD

Report 2200

CHEMICAL ANALYSIS FOR CHITIN AS A MEASURE OF FUNGAL INFILTRATION OF CELLULOSIC MATERIALS

December 1976

Approved for public release; distribution unlimited.

U.S. ARMY MOBILITY EQUIPMENT
RESEARCH AND DEVELOPMENT COMMAND
FORT BELVOIR, VIRGINIA

Destroy this report when no longer needed.
Do not return it to the originator.

The citation in this report of trade names of commercially available products does not constitute official endorsement or approval of the use of such products.

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER
2200

2. GOVT ACCESSION NO.

3. RECIPIENT'S CATALOG NUMBER

4. TITLE (and Subtitle)
CHEMICAL ANALYSIS FOR CHITIN AS A MEASURE OF FUNGAL INFILTRATION OF CELLULOSIC MATERIALS

5. TYPE OF REPORT & PERIOD COVERED
Final Report, December 1973 through July 1975

6. PERFORMING ORG. REPORT NUMBER
Dec 73 - Jul 75

7. AUTHOR(s)
Gertrud Ernst
Dario A. Emeric
Sidney Levine

8. CONTRACT OR GRANT NUMBER(s)

9. PERFORMING ORGANIZATION NAME AND ADDRESS
U.S. Army Mobility Equipment Research and Development Command, ATTN: DRDME-VC
Fort Belvoir, Virginia 22060

10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
PRON A1-4R0020-01-AW-EF
AMS No. 6858-49-11100
MTT Proj. AL-3-P6350-0-AW-EF

11. CONTROLLING OFFICE NAME AND ADDRESS
U.S. Army Mobility Equipment Research and Development Command
Fort Belvoir, Virginia 22060

12. REPORT DATE
December 1976

13. NUMBER OF PAGES
18

14. MONITORING AGENCY NAME & ADDRESS (If different from Controlling Office)
MERADCOM-2200

15. SECURITY CLASS. (of this report)
Unclassified

15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)
Approved for public release; distribution unlimited.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES
The work was accomplished by G. Ernst, D. A. Emeric, and S. Levine under the direction of Emil J. York, Chief, Material Technology Laboratory, U.S. Army Mobility Equipment Research and Development Command.

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)
Chitin Analysis
Fungal Infiltration
Cellulosic Materials
Fungus-Induced Deterioration
Glucosamine

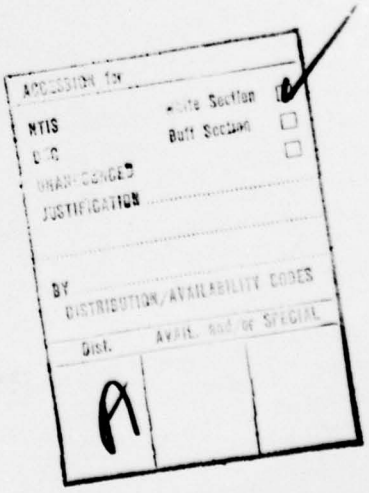
20. ABSTRACT (Continue on reverse side if necessary and identify by block number)
A chemical test method is described for indicating an early stage of fungus infiltration of cellulosic materials. The test method is based on mechanical separation (sonification) of fungus growth present in and on cellulosic materials and on the chemical analysis of chitin, a polymer of N-acetylglucosamine, which is a cell-wall constituent of most fungi.

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

UNCLASSIFIED

i SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

CONTENTS



CHEMICAL ANALYSIS FOR CHITIN AS A MEASURE OF FUNGAL INFILTRATION OF CELLULOSIC MATERIALS[1]

I. INTRODUCTION

1. **Statement of the Problem.** To provide a rapid and convenient chemical analytical technique which may be used for early detection of fungus infiltration of cellulosic materials.

2. **Background.** Cellulosic materials, e.g., cotton, jute, and paper, are widely utilized by the Army as end items or component parts in tropical and semitropical climates. Such environments provide optimum conditions for fungus growth and infiltration of these materials while in storage or in field use. An analytical chemical procedure has been developed to serve as a rapid and convenient preproduction control technique to determine the state of deterioration of certain items during long-term storage periods. In addition, this technique, which will detect fungal infiltration in its early stages, may be used as a diagnostic aid for investigating deterioration problems in the field. This procedure is based on the presence of chitin in the cell wall of most fungi[2] [3] which if determined quantitatively serves to indicate the degree of fungal surface growth and infiltration. It is a modification of a technique by Rondle and Morgan[4] [5] and is based on the reaction of amino sugars with acetyl acetone in an alkaline medium to form a chromogen that reacts with Ehrlich's reagent (paradimethylaminobenzaldehyde in a 1:1 mixture of ethyl alcohol and 12N hydrochloric acid) to produce a pink color. The intensity of this color is proportional to the concentration of the amino sugar.

II. EXPERIMENTAL PROCEDURE

3. **Approach to the Problem.** Carry out laboratory experiments to investigate variables as: shelf life of stock glucosamine, digestion time, consequences of interruptions or delays in test procedure step sequence, precision, sensitivity, sample preparation modifications of the original test procedures, etc.

---

[1] This report is based on material originally published by J. Ernst, D. A. Emeric, and S. Levine, "Chemical Analysis for Chitin as a Measure of Fungal Infiltration of Cellulosic Materials," *Textile Research Journal*, Vol. 46, No. 8, August 1976. Used by permission of the Textile Research Institute.

[2] Jackson W. Foster, *Chemical Activities of Fungi*, (New York: Academic Press, Inc., 1949), p. 90.

[3] V. W. Cochrane, *Physiology of Fungi*, (New York: John Wiley & Sons, Inc., 1958), pp. 39-43.

[4] L. E. Elson and W. T. J. Morgan, "A Colorimetric Method for the Determination of Glucosamine and Chondrosamine," Biochem. J. *27*: 1933, pp. 1824-1828.

[5] C. J. M. Rondle and W. T. J. Morgan, "The Determination of Glucosamine," Biochem. J. *61*: 1955, pp. 586-589.

Experimentally contaminate cotton or jute fabric squares with the following fungus cultures:[6]

a. *Aspergillus niger*, ATCC 10535, spores

b. *Chaetomium globosum*, ATCC 6205, spores

c. Expose jute burlap and cotton osnaburg samples to the U.S. Army Mobility Equipment Research and Development Command (MERADCOM) Tropical Testing Chamber environment.

d. Expose cotton osnaburg samples to soil burial in the MERADCOM Tropical Testing Chamber.

When growth becomes visible under the microscope but is not heavy enough to be seen with the naked eye, dislodge the growth with the ultrasonic probe and analyze for chitin (see figure).

III. RESULTS

4. Laboratory. Chitin (glucosamine) analyses were carried out on separate samples of paper, cotton osnaburg, and jute burlap, which were inoculated with *C. globosum* and *A. niger*, respectively. This approach was utilized to obtain the chitin content of known fungus species during various stages of maturity so as to obtain an indication of the method's sensitivity. The slower growing *C. globosum* appeared to be of greater value for this purpose, and, thus, *A. niger* was not utilized beyond the early stages of the investigation. The major concern of this analytical effort was that it be sensitive enough to chemically indicate fungus infiltration (at least 2-micrograms/sample area higher than a like sample area of the fabric blank) before it became visually detectable. It was soon evident that the cellulose fabric matrix provided sufficient interference to compromise the sought-after sensitivity. Several techniques were applied, in vain, to minimize this interference, such as: filtration through activated charcoal, filtration through 0.45-micrometer millipore filters, passage through cationic-exchange resins, treatment with hot caustic digestion prior to the hydrochloric acid digestion, and autoclaving prior to analysis.

A technique that was successful in minimizing matrix interference and at the same time increasing sensitivity was the dislodgment of the fungi from the fabric by means of an ultrasonic probe (sonification). It was found that utilization of this dislodging technique made it possible to significantly increase the matrix area per

[6] The fungus cultures were supplied by the Pioneering Research Laboratory, U.S. Army Natick Laboratories, Natick, Massachusetts, 01700. The present address for obtaining cultures is: G. E. Simmons, Natick Culture Collection of Fungi (QM), Department of Botany, University of Massachusetts, Amherst, Massachusetts, 01002.

PHOTO A
Prior to Sonification

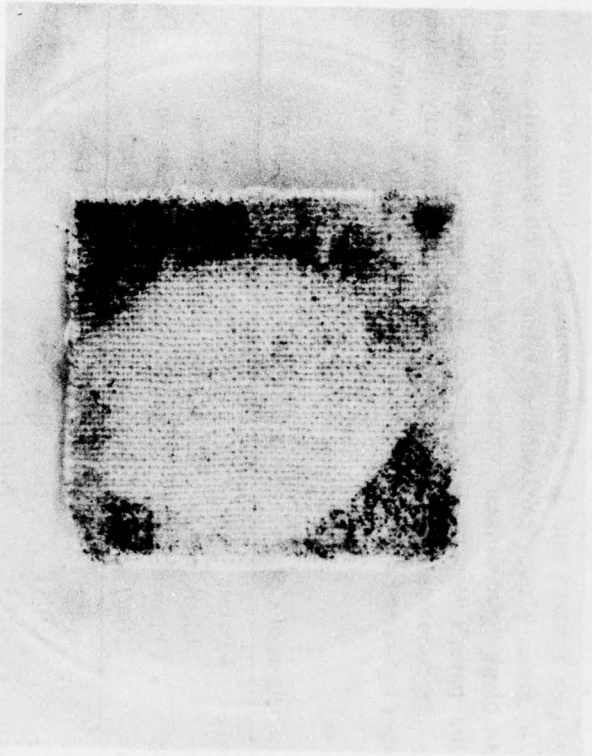


PHOTO B
After Sonification

Ultrasonic dislodgment of *C. globosum* from 5.1- by 5.1-centimeter cotton osnaburg fabric. Photo B shows area of dislodgment by sonic probe of visible growth specimen for illustration only.

sample over previous sample areas (6.5 to 26.0 cm^2) to a minimum of 260 cm^2 without any increase in interferences (sample blank), thus increasing the lower detection limits of the method (see Tables 1 and 2). Furthermore, the addition of the washing technique resulted in fabric blank "glucosamine" values which are quite similar in magnitude to reagent blank "glucosamine" values. Sheet paper was dropped from this program because the paper's structural integrity is destroyed during sonification, resulting in a pulp suspension which was difficult to separate from any dislodged fungus growth. Paper in knitted or woven form would undoubtedly be amenable to this technique.

Table 1. Analysis of Cotton Osnaburg for Chitin (Glucosamine)[a]

Sample No.[b]	Reagent Blank Chitin (μg)	Fabric Blank Chitin[c] (μg)	Visual Sample Appearance[d]	Chitin Sample (μg)	Net Diff. (μg)
1	6.0	14.3	No visible growth	18.5	4.2
2	6.5	9.5	No visible growth	17.2	7.7
3	6.5	10.5	No visible growth	18.0	7.5
4[e]	4.0	4.0	No visible growth	12.8	8.8
5	6.5	10.5	Light growth visible	18.5	8.0
6	6.0	14.3	Light growth visible	25.5	11.2
7	6.5	9.5	Light growth visible	20.8 (96 hours)	11.3

[a] These data represent the range of low-level chitin analyses of untreated cotton osnaburg on nonnutrient agar innoculated with *C. Globosum*, ATCC 6205.

[b] Sample area is 260 cm^2; incubation is 72 hours unless otherwise noted.

[c] Untreated cotton osnaburg – not inoculated with *C. globosum*.

[d] Under 36X microscope examination.

[e] Freshly distilled acetylacetone results in a reduced reagent blank; hence, a reduced fabric blank – the net difference between fabric blank and sample is quite similar, as shown in samples 3 and 4.

5. **Samples from Soil Burial.** Cotton osnaburg samples removed from a soil-burial environment were found to have high glucosamine levels. However, it was determined that the high glucosamine levels were caused by the soil (soil-containing interferences) which remained in the cotton after the soil-removal process.

6. **Samples from Tropical Testing Chamber.** Cotton osnaburg and jute burlap samples were exposed to the MERADCOM Tropical Testing Chamber environment for a period of 9 days. The exposed samples contained isolated growth that was not visible to the naked eye but was visible under the microscope. Chitin analyses of 260-cm^2 areas of the exposed fabrics gave positive indications of early stages of fungus growth.

Table 2. Analysis of Jute Burlap Fabric for Chitin (Glucosamine)[a]

Sample No.[b]	Incubation Time (hours)	Reagent Blank Chitin (μg)	Fabric Blank Chitin[c] (μg)	Visual Sample Appearance[d]	Chitin Sample (μg)	Net Diff. (μg)
1	70	8.0	8.4	No visible growth	13.2	4.8
2	72	8.0	8.4	No visible growth	11.5	3.1
3	72	4.5	4.8	Visible growth	10.3	5.5
4	96	4.0	6.0	Very light growth	8.3	2.3
5	96	4.0	6.0	Very light growth	11.5	5.5
6	158	4.5	4.8 (26 cm^2)	Visible growth	5.5	1.0
7	158	4.5	4.8 (52 cm^2)	Visible growth	6.8	2.0

[a] These data represent the range of low-level chitin analyses of untreated jute on nonnutrient agar inoculated with *C. globosum*, ATCC 6205.

[b] Sample area is 260 cm^2 unless otherwise noted.

[c] Untreated jute burlap – not inoculated with *C. globosum*.

[d] Under 36X microscope examination.

After the initial experiments, we concentrated our efforts on cotton osnaburg samples, which were experimentally contaminated by seeding them with a 1.0-milliliter spore suspension of *C. globosum*. The spore suspension was made from the yield of a culture on blotting paper placed on a nonnutrient agar plate.[7] The number of spores in the suspension was of no influence on the test results. The results of the chemical analyses and the visual appearances are correlated in Tables 1 and 2.

IV. DISCUSSION

7. **Discussion.** Experimental data in this paper show that it is possible to detect fungus infiltration in cellulosic materials, such as cotton and jute, before it becomes visible to the naked eye by a modification of the method by Rondle and Morgan (see appendix).

The application of an ultrasonic dismembrator to dislodge fungi from cellulosic sample surfaces virtually eliminated the high and erratic glucosamine values encountered in our early work. The digestion of the cellulosic materials along with the fungi gave in some cases higher glucosamine values for materials with no growth (fabric blank) than for a like quantity or area of fabric containing visible growth. With the

[7] $NaNO_3$, 3.0 grams; K_2HPO_4, 1.0 gram; $MgSO_4 \cdot 7H_2O$, 0.5 gram; KCl, 0.25 gram; Agar (Bacto), 18 grams; and distilled water to 1 liter.

dismembrator it was possible to "concentrate" fungal growth, with little or no fabric interference, to a level within limits of the established quantitative curve. This is similar in concept to concentrating a minute quantity of a substance in a fluid by evaporating a large quantity of this fluid to a much smaller volume.

V. CONCLUSIONS

8. **Conclusions.** Based on the work contained in this report, it is concluded that:

a. It is possible to detect low levels of fungus infiltration in cellulosic materials.

b. A rapid and convenient technique has been established to detect the presence of low levels of fungus infiltration in cellulosic materials even if they are heavily soiled or stained.

c. Fungus growth on fabrics after soil burial cannot be detected with this method because of significant interference from soil residues.

d. This method cannot be utilized for detection of fungus growth on paper because sonification disintegrates the paper, preventing effective separation of the fungi.

BIBLIOGRAPHY

Blumenthal, H. J., and S. Roseman, "Quantitative Estimation of Chitin in Fungi." J. Bact. *74:* 222-224, 1957.

Boas, N. F., "Method for the Determination of Hexosamines in Tissues." J. of Biol. Chem. *204:* 553-563, 1953.

Ernst, G., D. A. Emeric, and S. Levine, "Quantitative Chemical Assay of Chitin as a Measure of Fungal Infiltration in Cellulosic Materials." *Textile Research J.*, Vol. 46, No. 8, 1976.

Hubbell, D. H., Assistant Professor, University of Florida, Gainesville. Written Communication, June 3, 1971; August 14, 1972.

Mead, D. J., D. H. Hubbell, and B. N. Richards. "Quantitative Chemical Assay for Fungal Infection." Paper presented at the Annual Meeting of the American Society of Microbiology, Minneapolis, Minnesota, 1971.

Roseman, S., and J. Daffner. "Colorimetric Method for Determination of Glucosamine and Galactosamine." Anal. Chem. *28:* 1743-1746, 1956.

APPENDIX

ANALYTICAL PROCEDURE FOR THE DETERMINATION OF CHITIN AS GLUCOSAMINE

1. Equipment and Materials

a. Apparatus

(1) Spectrophotometer or colorimeter (wavelength: 530 nanometers)
(2) Heaters
(3) Refluxing apparatus (preferably with ground-glass joints)
(4) 10-milliliter test tubes graduated in 0.2-milliliter divisions with ground-glass stoppers
(5) Ultrasonic dismembrator
(6) Shaking apparatus

b. Reagents

(1) 12N hydrochloric acid
(2) 2N hydrochloric acid
(3) 0.5N hydrochloric acid
(4) 10N sodium hydroxide
(5) 1N sodium hydroxide
(6) Phenolphthalein solution (1 percent)
(7) 2, 4-pentanedione (acetylacetone). This reagent must be redistilled every 2 weeks.
(8) 0.5N sodium carbonate
(9) Ethanol (absolute)
(10) Ehrlich's reagent (1.336 grams of ρ-dimethylaminobenzaldehyde is dissolved in 50 milliliters of ethanol followed by the addition of 50 milliliters of 12N hydrochloric acid). Store at -10° C.
(11) Bushnell-Haas medium
(12) Glucosamine hydrochloride (1 milliliter = 8.6 milligrams glucosamine). Store frozen at -10° C.

2. **Procedure**

a. Take at least ten 5.1- by 5.1-centimeter specimens of the cellulosic fabric under consideration (cotton osnaburg, jute burlap) for each test sample. Place each square in a dish that is at least 5.1 centimeters wide and contains 20 to 25 milliliters distilled water.

b. Sweep back and forth over the sample surface at least three times with an ultrasonic dismembrator tip (Branson high-intensity sonic processor or similar) to dislodge the fungus material. Discard the matrix, and pour the liquid containing the fungus material into a graduated, 400-milliliter beaker. Repeat the ultrasonic technique for the remaining samples; combine the liquids of all samples in one graduated, 400-milliliter beaker. Place the beaker on a preheated hotplate, and let fibers settle. Aspirate the supernatent fluid carefully, leaving 40 milliliters in the beaker. Add 200 milliliters of distilled water to the beaker, and place it on the preheated hotplate. Repeat above fiber agglomeration step. A matrix control must be analyzed simultaneously.

c. Add 40 milliliters of 12N hydrochloric acid to the 40-milliliter volume and transfer to the refluxing apparatus.

d. Reflux for 6 hours.

e. Cool the contents and filter through a Whatman 41-H filter paper or equivalent. Collect the filtrate into a 100-milliliter volumetric flask.

f. Rinse with distilled water, and bring flask up to volume. (The analysis may be interrupted at this time for a maximum of 24 hours.) A reagent blank and glucosamine standard must be carried throughout the procedure.

g. A 1.0-milliliter aliquot is taken from the test solution(s) and pipetted into a 10-milliliter test tube(s). Analysis must be carried out at least in duplicate.

h. Add a drop of phenolphthalein, and slowly titrate with 10N NaOH until a pink color appears; then, back-titrate with 2N HCl until the pink color is discharged. Back-titrate with 1N NaOH until a pink color appears, and then back-titrate with 0.5N HCl until the pink color is discharged.

i. Pipet 1.0 milliliter of acetylacetone (1.0 milliliter of acetylacetone dissolved in 50.0 milliliters of 0.5N sodium carbonate). Prepare daily. The final volume at this point should be 3.0 ± .2 milliliters; bring to volume with distilled water if necessary.

j. Place a glass tube condenser into a test tube. The condenser must not touch the contents of the test tube. NOTE: An elongated glass tube with one sealed end or a conical test tube filled with water will act as a loose stopper and condenser to prevent the loss of acetylacetone.

k. Place the test tube containing the condenser in a vigorously boiling water bath for 20 minutes.

l. Bring the contents of the test tube to room temperature, and add ethanol (absolute) up to the 9.0-milliliter mark.

m. Add 1.0 milliliter of Ehrlich's reagent, and thoroughly mix the contents of the tube.

n. Place the test tube in a 65° C water bath for 10 minutes to accelerate the liberation of carbon dioxide. Bring the test tube to room temperature, and bring up to the 10-milliliter mark with alcohol if necessary.

o. Spectrophotometer is set to 100-percent T with a reagent blank at a wavelength of 530 nanometers.

p. Transfer contents to a spectrophotometer cell. Color must be read within ½ hour to avoid error due to fading.

DISTRIBUTION FOR MERADCOM REPORT 2200

No. Copies	Addressee
	Department of Defense
1	Director, Technical Information Defense Advanced Research Projects Agency 1400 Wilson Blvd Arlington, VA 22209
1	Director Defense Nuclear Agency ATTN: STTL Washington, DC 20305
12	Defense Documentation Center Cameron Station Alexandria, VA 22314
	Department of the Army
1	Commander, HQ TRADOC ATTN: ATEN-ME Fort Monroe, VA 23651
1	HQDA (DAMA-AOA-M) Washington, DC 20310
1	HQDA (DALO-TS M-P) Washington, DC 20310
1	HQDA (DAEN-RDL) Washington, DC 20314
1	HQDA (DAEN-MCE-D) Washington, DC 20314
1	Commander US Army Missile Research and Development Command ATTN: DRSMI-RR Redstone Arsenal, AL 35809
1	Chief, Engineer Division DCSLOG ATTN: AFKC-LG-E HQ Sixth US Army Presidio of San Francisco, CA 94129
1	Director Army Materials and Mechanics Research Center ATTN: DRXMR-STL Technical Library Watertown, MA 02172
2	Army Materials and Mechanics Research Center ATTN: DRXMR-M Watertown, MA 02172
1	U.S. Army Ballistic Research Laboratories Technical Library DRXBR-LB (Bldg 305) Aberdeen Proving Ground, MD 21005
1	Commander Edgewood Arsenal ATTN: SAREA-TS-L Aberdeen Proving Ground, MD 21010
1	Commander US Army Aberdeen Proving Ground ATTN: STEAP-MT-U (GE Branch) Aberdeen Proving Ground, MD 21005
1	Director US Army Materiel Systems Analysis Agency ATTN: DRXSY-CM Aberdeen Proving Ground, MD 21005
1	Director US Army Engineer Waterways Experiment Station ATTN: Chief, Library Branch Technical Information Center Vicksburg, MS 39180
1	Commander Picatinny Arsenal ATTN: SARPA-TS-S No. 59 Dover, NJ 07801
1	Commander US Army Troop Support and Aviation Materiel Readiness Command ATTN: DRSTS-KTE 4300 Goodfellow Blvd St. Louis, MO 63120

No. Copies	Addressee
2	Director Petrol & Fld Svc Dept US Army Quartermaster School Fort Lee, VA 23801
1	Commander US Army Electronics Research and Development Command ATTN: DRSEL-GG-TD Fort Monmouth, NJ 07703
2	Engineer Representative US Army Standardization Group, UK Box 65, FPO New York 09510
1	Commander Rock Island Arsenal ATTN: SARRI-LPL Rock Island, IL 61201
1	Commander Frankford Arsenal ATTN: Library, K2400, B1 51-2 Philadelphia, PA 19137
1	Learning Resources Center US Army Engineer School Bldg 270 Fort Belvoir, VA 22060
1	Commander Headquarters, 39th Engineer Battalion (Cbt) Fort Devens, MA 01433
1	President US Army Armor and Engineer Board ATTN: ATZK-AE-TD-E Fort Knox, KY 40121
1	Commandant US Army Command and General Staff College ATTN: ATSW-RI-L Fort Leavenworth, KS 66027
1	Commander 2nd Engineer Group ATTN: S4 APO San Francisco 96301
1	Commander and Director USAFESA ATTN: FESA-RTD Fort Belvoir, VA 22060

No. Copies	Addressee
1	U.S. Army Electronics Proving Grounds ATTN: Miss E. Collanto Fort Huachuca, AZ 85613
	MERADCOM
1	Commander Technical Director Assoc Tech Dir/R&D Assoc Tech Dir/Engrg & Acq Assoc Tech Dir/Matl Asmt Assoc Tech Dir/Tech Asmt CIRCULATE
1	Chief, Countermine Lab Chief, Energy & Water Resources Lab Chief, Electrical Power Lab Chief, Camouflage & Topographic Lab Chief, Marine & Bridge Lab Chief, Mechanical & Construction Eqpt Lab Chief, Counter Intrusion Lab Chief, Product Assurance & Testing Lab Chief, TARSO CIRCULATE
5	Material Technology Lab
25	Chemistry-Biodeterioration Rsch Grp
3	Technical Reports Ofc
3	Security Ofc
2	Technical Library
1	Requirements & Programs Ofc
1	Public Affairs Ofc
1	Legal Ofc
	Department of the Navy
1	Director Naval Research Laboratory ATTN: Code 2627 Washington, DC 20375
1	Commander, Naval Facilities Engineering Command Department of the Navy ATTN: Code 032-A 200 Stovall St Alexandria, VA 22332
1	US Naval Oceanographic Office Library (Code 1600) Washington, DC 20373

No. Copies	Addressee
	Department of the Air Force
1	HQ USAF/RDPS (Mr. Allan Eaffy) Washington, DC 20330
1	AFSC/INJ Andrews AFB, MD 20334
1	AFCEC/XR/21 Tyndall AFB, FL 32401
1	HQ USAF/PREES ATTN: Mr. Edwin B. Mixon Bolling AFB-Bldg 626 Washington, DC 20332
1	AFAPL/SFL Wright-Patterson AFB, OH 45433
	Others
1	Professor Raymond R. Fox School of Engineering and Applied Science The George Washington University Washington, DC 20052